

## Using local micro-biota to extract biodegradable plastics from food waste through a natural fermentation process

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**Summary.** The main problems of mankind in recent decades are the accumulation of various industrial, agricultural, and food production wastes. Their ineffective disposal and management practices have a detrimental effect on human health and cause environmental pollution, which requires urgent action. Food waste has become a complex phenomenon lately, attracting the attention of scientists, consumers, and activists. This study aims to apply the biotechnology of converting food waste into crystals of polylactic acid (PLA), a monomer for biodegradable plastic. A food waste sample is taken from the student canteen; wash, to remove impurities and fermentation of carbohydrate waste by autotrophic lactic acid bacteria in a natural process for about seven days in the optimal temperature range. Finally, lactic acid molecules polymerized by condensation reaction to form poly L-lactic acid (PLA) crystals, and then a biodegradable bioplastic.

**Keywords:** food waste, fermentation, lactic acid, poly L-lactic acid (PLA), bioplastics, biodegradable, polymerization

### Introduction

Plastics are known to be more useful than many other materials because of their lightness, excellent processability, economy, therefore they are more profitable when choosing materials in many industrial and commercial proposals. In the twentieth century, the production of most traditional plastics from petroleum was widely used [1]. But these petroleum-based plastics are causing serious environmental problems around the world because they are not biodegradable [2]. For this reason, the use of such plastics is prohibited in many countries around the world.

Recently due to shortages petroleum and environmental concerns have intensified the innovation of new cost-effective, greener alternatives [3]. Thus bio-based and biodegradable polymers have emerged as novel alternatives that have the peculiar ability to be ultimately converted into biomass and harmless by-products through the action of microbes [4]. The so-called green polymers can offer sustainable development in the economy and ecology [3], preserving fossil-based raw materials, and minimizing the volume of plastic garbage via decomposition in the natural cycle contributing to climate protection [5].

Food waste implies simply foods intended for consumption but are discarded and cannot be used, and the term 'food losses' mostly refer to the decrease in the amount of food staff throughout the food supply chain which is supposed to be used for human consumption [6]. In many countries in addition to plastic waste, food waste also has become a common occurrence due to lack of storage technologies, out of dates, food prepared for

a group of people in colleges, marriage parties, and other festivals. It has increased even more after the start of the buffet system, period of competition, and ostentation. When food waste is dumped, it decomposes and causes a bad odor to the surrounding, it pollutes water, land, and climate (contributes to increase in greenhouse gas emissions, GHG) as well as biodiversity [7], many times animal deaths or ill are reported due eating the rotting food. Therefore converting food wastes to bio-based organic products such as biogas, bio-fertilizer, or PLA (bioplastic) is a double advantage, because it leads to lowering GHG emissions, minimizing the amount of non-biodegradable plastics, and decreasing the accumulation of food garbage in landfills.

Polylactic acid (PLA), one of the most promising is bio-compatible thermoplastic aliphatic polyester. It is produced by the polymerization of lactides. PLA is used for many different applications, from packaging to agricultural products and disposable materials, as well as, surgery and pharmaceutical fields [8, 9]. More ever PLA and its copolymers which have been developed as biomedical materials were based on their bio-absorbability and bio-compatibility nature and are been used in many therapeutic and pharmaceutical fields such as drug delivery systems, protein encapsulation, and delivery development of microspheres and hydrogels [9, 10].

Lactic acid (2-hydroxy propionic acid) is the basic building block for PLA. It is a highly water-soluble, three-carbon chiral acid that is naturally occurring and is most commonly found in the L (–) isomer form. It has a chemical formula of  $\text{CH}_3\text{CHOHCOOH}$ , and its chemical name is the commonly occurring hydroxycarboxylic acid.

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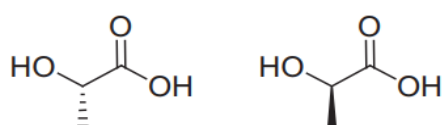
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It was first discovered in 1780 by the Swedish chemist Scheele [11]. Lactic acid is found naturally as an organic acid and can be produced either by fermentation or chemical synthesis. In the case of fermentation condition, a pure form of lactic acid can be obtained however in chemical production it is a racemic mixture [12]. It is present in many foods both naturally and as a product of in-situ microbial fermentation. The lactic acid bacteria generally include several classes in terms of cell morphology, genus *Leuconostoc*, *Lactococcus*, *Lactobacillus*, *Pediococcus*, *Enterococcus*, *Streptococcus*, *Vagococcus*, *Aerococcus*, *Carnobacterium*, *Tetragenococcus*, *Oenococcus*, and *Weissell* [12, 13], most of them produce L-lactic acid while few produce D – or DL-lactic acids. Fermentation can be carried out in batch or continuous type [14, 15].



L-lactic acid

D-lactic acid

Figure 1. Three-dimensional structure of L(+) – lactic acid and D(–) – lactic acid

### Materials and Methods

Airtight container (3L), muslin cloth, pH meter, thermometer, electronic balance, measuring cylinders and centrifuge machine, test tubes, Bunsen burner, test tube holder, Petri-dishes, beakers, biuret, conical flask, spatula pipet, refrigerator, inoculation loop, incubation, Sulphuric acid ( $\text{H}_2\text{SO}_4$ ), Calcium hydroxide ( $\text{Ca}(\text{OH})_2$ ), Methanol, Distilled water, Hydrochloric acid ( $\text{HCl}$ ), chocolate agar media, anti-bumping granules.

Two kilograms of rice waste were collected from the student cafeteria waste collector and washed to remove dirty components such as oil, spices, and others, then it was mixed with two liters of distilled water in a plastic container with a tight cover (figure 2.). Its initial PH was recorded and allowed to be fermented (batch fermentation Process) in an open environment for one week (seven days), within a temperature range of 30–35 °C [16].

During the fermentation process, the pH was regularly measured using a pH meter at 6 pm each day (table 1). When the pH meter showed constant reading after the fifth day for the next two days, filtration was carried out using a muslin cloth. The solid residue containing the undigested rice dead cells and other impurities were discarded. The broth was then treated with 310 ml Calcium hydroxide (Chem. Rxn 1) and stored at 4° C for

3 days [16]. The solution was poured into test tubes and was centrifuged at 3500 rpm for 15 minutes. The liquid part was discarded from each test tube and the precipitate (solid part) in each test tube was dissolved by Methanol. The solution from each test tube was collected into one large beaker and subsequently, hydrolysis was carried out using 70 %  $\text{H}_2\text{SO}_4$ , (chem Rxn 2) to obtain lactic acid (liquid) and calcium sulfate (precipitate) and stored at 4 °C for 3 days, for more precipitate formation. Thereafter it was distributed in test tubes and then centrifuged at 3000 rpm for 15 minutes to separate the calcium sulfate (solid ppt.) which was discarded, and liquid, (methanol and lactic acid solution) (chem. Rxn 3). The solution was boiled at 100 °C remove methanol and water leaving lactic acid alone. Pure lactic acid was collected measured and was tested for its chemical and physical properties, such as pH, boiling point, and concentration. Finally, lactic acid was polymerized to form crystals of polylactic acid crystals (figure 2.) [17, 18].

Table 1.

Caption

Days	pH
Day 0 (initial)	6.25
Day 1	6.21
Day 2	5.50
Day3	5.10
Day 4	4.50
Day5	3.84
Day 6	3.84
Day 7	3.84



Figure 2. Bio-reactor plastic container



Figure 3. Polymerization of lactic acid 10:58 table 1. PH measures

## Result and discussion

Lactic acid was the main organic acid produced ( $\approx 98\%$ ) from starchy food waste fermentation by a lactic acid-producing group of bacteria followed by minor impurities such as acetic acid and formic acid and carbon dioxide, provided optimum parameters are maintained  $30^{\circ}\text{C}$  to  $40^{\circ}\text{C}$  and pH of 6.0 to 6.5 that promotes the growth of lactic acid-producing bacteria [19]. During the fermentation process, the pH was dropped from the initial 6.21 to 3.84, until the fifth day when it came to be constant (3.84 pH) for the next two days (table 1). This gradual drop of pH was due to the formation of organic acid as a result of the metabolic activity of the microorganisms using the substrate sugar from rice. A similar trend was also obtained by Sakai and Ezaki (2006), whereby lactic acid was the predominant organic acid produced in kitchen waste fermentation. Calcium hydroxide was added gently to 1.5 l broth to form calcium lactate precipitate (*Chemical reaction 1*) and simultaneously pH was measured upon the addition of Calcium hydroxide to obtain the average pH of calcium lactate around 6. The solution was stored in a refrigerator at  $4^{\circ}\text{C}$  for three days to get good precipitated [16]

The calcium lactate solution was poured into several test tubes and was centrifuged at 3500 rpm for 15 minutes just to get a good solid precipitate of calcium lactate; this precipitate (solid part) in each test tube was dissolved by Methanol which is known as the *esterification process*. The addition of 70 %  $\text{H}_2\text{SO}_4$  (hydrolysis) (*Chemical reaction 2*) lead to the production of lactic acid (liquid) and by-product calcium sulfate (precipitate) after storage at  $4^{\circ}\text{C}$  for 3 days and centrifugation at 3000 rpm for 15 minutes concentrate precipitate was formed.

Chemical reaction 1.  $2\text{C}_2\text{H}_5\text{COOH} + \text{Ca}(\text{OH})_2 \rightarrow (\text{C}_2\text{H}_5\text{COO})_2\text{Ca} + \text{H}_2\text{O}$

Chemical reaction 2.  $(\text{C}_2\text{H}_5\text{COO})_2\text{Ca} + \text{H}_2\text{SO}_4 \rightarrow 2\text{CH}_3\text{CHOHCOOH (aqua)} + \text{CaSO}_4$

The solution was boiled at  $100^{\circ}\text{C}$  to remove methanol (B.pt.  $78^{\circ}\text{C}$ ) and water (B.pt.  $100^{\circ}\text{C}$ ) leaving lactic acid alone (B. Pt.  $122^{\circ}\text{C}$ ). Finally, 256ml lactic acid is obtained from the two kilograms

of rice waste and was polymerized using a *condensation reaction* (Chemical reaction 3) to form polylactic acid crystals[20].

Chemical reaction 3.  $2\text{CH}_3\text{CHOHCOOH} \rightarrow \text{C}_6\text{H}_8\text{O}_4 + \text{H}_2\text{O}$  (polymerization)

There were slight deviations for some of the chemical and physical standard parameters, such as pH, boiling point, and concentration. This fluctuation could be due to substrate characteristics, pH, and temperature fluctuations.

## Conclusions

Kitchen wastes comprise a lot of active indigenous microbes where it plays significant roles in converting large molecules into simpler molecules. In this study, lactic acid fermentation with indigenous bacteria was investigated using batch fermentation with intermittent pH adjustments to 6 and 8 at ambient temperature  $30\text{--}35^{\circ}\text{C}$ . PLA is being used as raw material for the production of bio-plastics not solely because of its biodegradability but also is made from renewable resources. PLA is in both durable and short-term applications. Increasing demand for lactic acid and research on eco-friendly polylactic acid production has made it necessary to develop a sustainable and conventional method for lactic acid production by fermentation. By now it is obvious that under controlled environmental conditions like at pH temperature substrate etc. the productivity of lactic acid by fermentation can be increased and thus take this advantage to produce biodegradable plastics all over the planet.

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#### **Contribution**

**Merhawi K. Tsegay** consultation during the study

**Lyudmila T. Sukhenko** wrote the manuscript, correct it before filing in editing and is responsible for plagiarism

#### **Conflict of interest**

The authors declare no conflict of interest.

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